

Studies on the Nodule Bacteria of *Astragalus sinicus* (Genge).

By

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This paper deals with some physiological studies together with the inoculation experiment on the nodule bacteria of *Astragalus sinicus* (Genge) which is the most important green manure used for the paddy-field in Japan.

In this investigation, the following aspects of the organism were undertaken and the results are reported :

1. The relation between the rate of migration of organism and the moisture content of the soil.
2. The chemotaxic nature of the organism.
3. The influence of various concentration of sodium chloride on the growth of the organism.
4. The inoculation experiments in both the pots and the field.

I. The relation between the rate of migration of organism and the moisture content of the soil.

It is interesting to ascertain the rate of migration of the organism in the soil of various moisture contents since Genge is cultivated in the paddy-field of which the soil contains a large amount of water usually. The experiment was carried out in the following manner :

500 g. of washed quartz sands (0.5—1.0 mm.) were placed in a Petri dish (15 cm. diameter), and various amount of the mannit culture solution was added to reach the moisture content desired. The cover of Petri dish was provided with a filter paper to prevent the moisture condensation. To measure the distance of migration of the organism, a glass rod with the measurement is placed in each Petri dish diametrically, and the inoculation was made at one end and the samples for examination for the presence of organism were taken from each known distance as indicated in the table, at 24 hours intervals. The test for the presence of organism was made by both culturally and microscopically. The results are given in Table I.

Table I.
Rate of Migration of Genge Nodule Bacteria and
Moisture Content.

Distance cm. % Moisture.	1	2	3	4	5	6	7	8	9	10	11	12	13
26	24	24	48	48	72	72	72	96	96	96	96	96	96
25	24	24	48	48	72	72	72	72	72	96	96	96	96
24	24	24	48	48	72	72	72	96	96	96	96	96	96
23	24	24	48	48	48	72	72	72	72	96	96	96	96
22	24	24	48	48	72	72	72	96	96	96	96	96	96
21	24	24	48	48	72	72	72	96	96	96	96	96	96
20	24	24	48	48	72	72	72	72	72	72	96	96	96
19	24	24	48	48	48	72	72	72	72	72	96	96	96
18	24	24	48	48	48	72	72	72	72	72	72	72	72
17	24	24	48	48	48	48	48	72	72	72	96	96	96
16	24	24	48	48	72	72	72	72	72	72	96	96	96
15	24	48	48	48	72	72	72	96	96	96	120	120	120
14	24	48	48	48	72	72	72	72	96	96	120	120	120
13	24	48	48	48	72	72	72	72	96	96	120	120	120
12	24	48	72	96	96	96	96	120	120	120	144	144	144
11	24	48	72	96	96	120	120	120	120	144	144	144	144
10	24	48	48	72	96	96	96	120	120	120	144	144	144
9	24	48	48	72	96	120	144	144	168	168	168	168	168
8	24	48	72	96	120	144	168	168	168	192	192	192	192
7	48	72	120	192	240	264	288	—	—	—	—	—	—
6	48	72	—	—	—	—	—	—	—	—	—	—	—
5	—	—	—	—	—	—	—	—	—	—	—	—	—

Note: The figure in the table indicates the number of hours.

The rate of migration of the organism is influenced very much as shown in the table by the moisture content in the soil. According to the results obtained in this investigation, 18% moisture seems to be the optimum, and below 5% no migration was observed. It was investigated as high as 26% moisture since the saturation capacity was 27%. Judging from these results, the condition of paddy-field is well suited for the Genge nodule culture.

II. The chemotaxic nature of the Genge nodule bacteria.

It was experimented to ascertain if the Genge nodule bacteria have a positive or negative chemotaxic action against the germinating Genge seeds, the extract of Genge seeds and also of wheat seeds. The same experimental procedure was employed as in case of the previous experiment and the results are given in Table II.

Table II.
Chemotaxic Action of Genge Nodule Bacteria.

Kind.	Treatments. Distance cm. No. of Expt.	Seeds.		Germinating seeds.		Seed extract.	
		5	10	5	10	5	10
Control.	1	48	72	48	72	48	72
	2	48	72	48	72	48	72
	3	72	96	48	72	48	72
	4	48	72	72	96	48	72
Genge.	1	48	72	48	48	48	96
	2	48	72	24	48	48	72
	3	48	72	24	48	48	72
	4	48	72	48	48	48	72
Wheat.	1	48	72	48	72	48	72
	2	48	72	48	72	48	72
	3	48	96	48	72	48	72
	4	72	96	48	96	48	96

Note: The figure in the table indicates the number of hours.

As the results indicate, it is clearly noted that Genge nodule bacteria have the positive chemotaxic action toward the Genge seeds especially the germinating seeds. Toward the germinated seeds, the organism almost doubled the speed over the others.

*III. Influence of various concentration of sodium chloride
on the growth of the organism.*

It is important to know the influence of salt content in the soil on the nodule bacteria since an extensive area of the Japanese paddy-field is the reclaimed soil and naturally contains a large amount of salt as it was reported previously¹⁾.

1.) A. ITANO and A. MATSUURA, *Berichte des Ohara-Inst. etc.*, V: 171—176, 1931.

The seawater (2.922% salt content, P_H 7.91) was tried first and found no growth of the organism. But an addition of the following constituents supported the growth:

Tricalcium phosphate	1 g.
Potassium sulfate	1 g.
Magnesium phosphate	1 g.
Ferric chloride	trace.
Cane sugar	10 g.
Agar	20 g.
Seawater	1,000 cc.

Again in the above medium the distilled water was substituted in place of seawater and various amount of salt (0.01—5.0%) as indicated in the table, was added to see the effect. The results are noted in Table III.

Table III.
Influence of Salt Concentration on the Growth
of Nodule Bacteria.

Kind.	No.	Control.	Concentration of salt. (%)												
			0.01	0.1	0.5	0.8	1.0	1.5	2.0	2.5	3.0	3.5	4.0	5.0	
Genge.	I	++	###	##	##	##	++	++	++	+	+	+	+	—	
	II	++	##	##	##	++	++	++	+	+	+	+	—	—	
Hubam clover.	I	+	##	##	##	++	++	++	+	+	+	+	—	—	
	II	++	##	##	##	++	++	++	+	+	+	—	—	—	

Note: (+) Growth; (++) Better growth; (###) Best growth; (—) No growth.

Table III indicates that for Genge nodule bacteria, above 4.0% salt content is detrimental and the optimum seems to be 0.01—0.5%; and for Hubam clover nodule bacteria, above 3.5% is detrimental and the optimum is the same as for Genge.

IV. Inoculation experiments in both the pots and the field.

It was investigated in both the pots and the field to ascertain the manure value of inoculation of Genge with the nodule bacteria.

A. Pot experiment:

In the pots, the influence of moisture content and also the effect of the soil in which Genge has been grown previously, have been investigated in the following manner.

A number of pot (26 inch diameter and 36 inch deep) was filled with 15 Kg. of the air-dried soil from the paddy-field or dry-farm, having 64% and 54% water holding capacity respectively, and 15 g. CaCO_3 , 5 g. calcium superphosphate, 6 g. wood ash were added to each pot a day before sowing the seeds. The moisture content was adjusted varying from 25–65% in the paddy-field soil keeping the control at 35–45%; and 25–55% in the dry-farm soil as indicated in Table IV and V. One gram of seeds was planted in each pot and was inoculated with the bacterial suspension which was prepared by taking two agar slant cultures and suspended in 300 cc. of sterile water, and covered with soil. The pots were looked after in the glass and net house according to the weather, by keeping track of the water supply dailey. The plants were sprayed twice with kerosene emulsion against aphids. The seeds germinated four days after the planting, and the growth condition was examined at five days intervals and noted in the tables.

Table IV.
Experiment in Paddy-field Soil.

% Moisture.	Days for germination.	Number of days after planted.									
		5	10	15	20	25	30	35	40	45	50
Control. {	35	3	4	4	4	5	4	4	4	5	4
	45	2	2	2	3	4	3	3	4	4	3
	25	2	2	3	2	2	2	1	1	2	1
	53	1	2	3	2	3	2	1	1	1	1
	54	2	1	1	1	1	1	2	2	3	2
	55	2	4	4	4	5	3	3	3	4	3
	65	3	5	—	—	—	—	—	—	—	—

Table V.
Experiment in Dry-farm Soil.

% Moisture.	Days for germination.	Number of days after planted.									
		5	10	15	20	25	30	35	40	45	50
Control. {	35	3	3	3	4	3	3	3	3	2	2
	45	2	4	4	—	—	—	—	—	—	—
	25	2	1	1	1	1	1	1	1	1	1
	35	2	2	2	2	1	1	1	1	1	1
	45	2	3	3	3	2	2	2	2	2	3
	55	3	4	—	—	—	—	—	—	—	—

Note: The figure in the table indicates the growth condition.

As the above tables indicate, in the paddy-field soil, the germination took place best in 45% moisture at the end of five days but the growth as a whole was the best in 35% moisture while in the dry-farm soil, 25% moisture was the best in all respects. The inoculated pots were much better than the control and the difference became much more evident as the growth progressed, and it was so especially in the dry-farm soil.

Fifty two days after the planting, the damage by aphid became so great that the further observation was abandoned and the following observations were made by taking the representative five plants from each pot in the paddy-field series and ten plants from the dry-farm series, for the total weight, the nitrogen content and the number of nodules formed. The results are given in Table VI, A and B, Table VII, A and B.

Table VI.
Crop of Genge harvested and Nitrogen Contents.

A. Paddy-field Soil.

% Moisture. Crop.	Control.		25	35	45	55
	35	45				
Crop. (g.)	0.347	0.484	2.410	3.576	1.363	0.658
Total nitrogen (g.)	0.0111	0.0169	0.0903	0.1289	0.0532	0.0189
% Total nitrogen.	3.192	3.485	3.745	3.605	3.902	2.873

B. Dry-farm Soil.

% Moisture. Crop.	Control.	25	35	45
	35			
Crop. (g.)	1.563	9.986	8.980	0.838
Total nitrogen (g.)	0.0423	0.3797	0.3244	0.0248
% Total nitrogen.	2.705	3.803	3.613	2.956

(See Table VII on next page.)

These tables indicate that much better results, in all respects, were obtained by inoculation. That is, by inoculating, the weight of crop in the inoculated pot of the same moisture content gave several times as much as that of the control, and also the number of nodules and the nitrogen content were more than those of the control. It is true with both kinds of soils.

As to the relation of the water content to the growth of Genge, 30% in the paddy-field soil and 25% in the dry-farm soil were the best or in term of the water

holding capacity of these soils 55% and 48% were the best respectively. Consequently it may be stated in general that 50% moisture content is best adapted for growing Genge.

Table VII.
Number of Nodules on Genge Roots.

A. Paddy-field Soil.

% Moisture.	No. of test.						Total number.	Average per plant.
		1	2	3	4	5		
Control.	35	11	16	26	5	13	71	14.2
	45	13	14	11	29	10	77	15.4
	25	28	30	26	64	21	169	33.8
	35	31	29	36	39	33	168	33.6
	45	34	23	24	20	35	136	27.2
	55	16	19	35	27	25	122	24.4

B. Dry-farm Soil.

% Moisture.	No. of test.											Total number.	Average per plant.
		1	2	3	4	5	6	7	8	9	10		
Control.	35	5	12	7	8	16	26	5	13	10	11	113	11.3
	25	14	19	29	24	16	37	26	32	62	53	312	31.2
	35	30	23	16	27	49	32	14	49	37	44	321	32.1
	45	14	20	21	15	22	7	11	5	17	19	151	15.1

B. Inoculation experiment in the field.

Since a marked good influence of inoculation was observed in the pot experiment, it was tried in the experimental field at this institute in which Genge was cultivated in a previous season.

- a.) Inoculation experiment in the field where Genge was grown in a previous year.

Four adjacent plots of 1 are each were taken and treated as indicated in Table VIII. Five hundred grams of seeds which have been rubbed with the sand, were used for each plot. For the inoculation, the bacterial suspension in 1% sugar solution was used and inoculated as usual. The seeds were sowed as in practical farming or broad casting and no special care was given until harvested on May 21st. The results are given in Table VIII.

Table VIII.
Crop of Genge harvested.

Plots.	Crop per plot.
Inoculated.	(Kg.) 131.2
Control.	123.7
"	120.0
Inoculated.	154.0

The results indicate that by inoculation, the crop was increased even in these plots where Genge was grown in the previous season so that the nodule bacteria may be present in sufficient number. Although the difference between the inoculated and uninoculated was not so great in this case, it is evident that the inoculation brought about the better crop.

b.) Field inoculation experiments under different treatment.

After the rice crop was harvested, eight ares of the field were divided into two parts, four ares each, and the one part was ploughed and the other left unploughed, and planted with the seeds which have been subjected to the following treatments: 1. soaked in water for three days; 2. rubbed with sand; and controlled with untreated seeds. The results are shown in Table IX.

Table IX.
Crop of Genge harvested under Various Treatments.

Plots.	Unploughed section.	Ploughed section.
	(Kg.)	(Kg.)
1.	742.0	855.0
2.	742.0	905.5
3.	676.0	1,198.0
4.	676.0	1,594.0
5.	676.0	1,651.0
6.	758.0	1,826.0

- Notes: Plot 1. Control (no inoculation and no treatment);
 2. Inoculation and no treatment.
 3. Soaked in water, no inoculation.
 4. " " " , inoculated.
 5. Rubbed, no inoculation.
 6. " , inoculated.

The figure in the table indicates the weight of green crop.

The data indicate that a largest crop was obtained from the ploughed section which was planted with the rubbed seeds, and among others, the crop in the ploughed section was much better than that of the unploughed section in general. In both cases, the inoculation gave better crop over the uninoculated section.

Summary:

From the results obtained in this investigation, the following summary may be made:

1. The rate of migration of the Genge nodule bacteria in the soil is influenced very much by the moisture content of the soil, and 18% seems to be the optimum; and below 5%, no migration.
 2. The Genge nodule bacteria have the strong positive chemotaxic action toward the Genge seeds especially to the germinating seeds.
 3. As to the concentration of salt, 0.01—0.5% seems to be the optimum for the Genge nodule bacteria and above 4.0% is detrimental.
 4. The beneficial influence of inoculation in both the paddy-field and dry-farm was noted even where the Genge has been grown previously.
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